

PRELIMINARY PHARMACOBIOLOGICAL STUDY  
OF NEW STEROIDAL SAPOGENINS

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**SUMMARY.** — *The chemotherapeutic study of a limited series of steroidal sapogenins from several endemic species of the flora of the Canary Islands is presented here. On the whole, they possess a very weak antibacterial activity, a slight antifungal effect and one of them, vespertilin, displays interesting cytostatic activity ( $ID_{50}=5 \mu\text{g/ml}$ ). A pharmacodynamic screening carried out on this product mainly revealed very slight toxicity, antihistaminic activity and a light tranquillizing effect. The data obtained justify further research.*

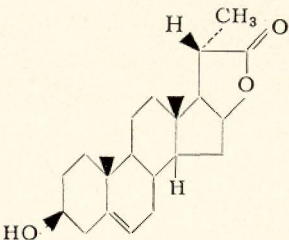
**RIASSUNTO.** — *Viene riportato lo studio chemioterapico di una serie limitata di sapogenine steroidee ottenute da diverse specie endemiche della flora delle Isole Canarie. Nel complesso, tali sostanze possiedono una debolissima azione antibatterica, un lieve effetto antifungino e una di esse, la vespertilina, anche un'interessante azione citostatica ( $DI_{50}=5 \mu\text{g/ml}$ ). Uno screening farmacodinamico effettuato con questo prodotto ha rivelato che la sostanza ha una tossicità trascurabile, e possiede sia attività antiistaminica che un lieve effetto tranquillante. I dati ottenuti giustificano ulteriori indagini.*

### Introduction

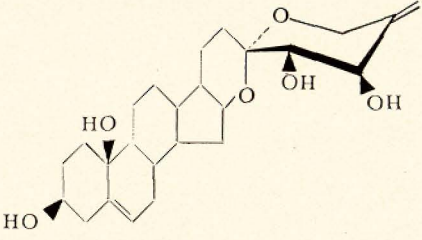
Steroidal sapogenins are a class of compounds amply distributed throughout the vegetable world. Although their therapeutic importance and their application as starting material in the synthesis of different types of medicinal drugs, mainly hormonal in nature, have been well known for some years (14), their chemotherapeutic potential has only recently been investigated and reported (12, 17). Within a wider



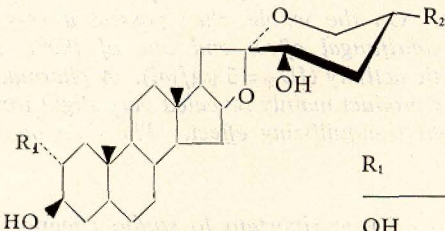
research project designed to detect possible pharmacological activity in a large series of new natural products isolated from species of the Spanish flora, we have carried out a preliminary study of this reduced series of spirostanic and lactonic steroidal sapogenins, the structures of which are shown in Fig. 1. They have been isolated for the first time from several highly representative species of the Macaronesian flora, some of which are widely applied in phytotherapy (1, 3).



Vespertilin

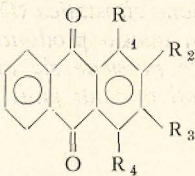


Dracogenin



Crestagenin  
Isoplexigenin B

R <sub>1</sub>	R <sub>2</sub>	5.6
OH	—CH <sub>2</sub> OH	—
H	—CH <sub>3</sub>	—



(V)	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
(VI)	OH	—CH <sub>3</sub>	H	OH
(VII)	OH	H	—CH <sub>3</sub>	H
	OH	—CH <sub>3</sub>	H	—OCH <sub>3</sub>

(Fig. 1)



## Material and methods

### 1) Species and compounds studied

a) *Dracaena draco* (L.) (Agavaceae), commonly known as "drago", from whose bark, collected in May in the outskirts of La Laguna (Tenerife), dracogenin, possibly one of the most oxidised steroidal genins ever isolated, was obtained, closely following a method previously reported by us (6).

b) *Isoplexis canariensis* (L.) Loud. (Scrophulariaceae), known as "cresta de gallo", the leaves of which, collected in Las Mercedes (Tenerife) in June, afforded, according to our method (4), a spirostane sapogenin, crestagenin, together with several anthraquinonic compounds (Fig. 1), all of which were studied.

c) *Semele androgyna* (L.) Kunth (Liliaceae), popularly called "gibalbera", from the aerial part of which, collected in May in Las Mercedes (Tenerife), isoplexigenin B, a lactonic steroid initially isolated from *Isoplexis sceptrum* (L.) Steud. (8) was obtained.

d) *Solanum vespertilio* (Ait.) (Solanaceae), or "rejalgadera", from whose fruitbodies collected in the mountains of Anaga (Tenerife) and adopting the described method (7), a steroidal lactone, vespertilin, was isolated.

All the products obtained were identified by comparison with authentic samples.

### 2) Measurement of cytostatic activity

Cytostatic activity was determined by measuring the inhibition of the development of a single-layer culture of HeLa 229 cells, starting at an initial concentration of approximately  $10^5$  cell/ml, in "TC Minimal Medium Eagle Dried". The products to be tested were suspended in Tween 80. A blank control and a positive control with 6-mercaptopurine were carried out under identical conditions, following the method described by Geran *et al.* (5). Each test was run three times and the readings were taken by microscopic methods after 72 h of incubation.

### 3) Determination of antimicrobial activity

The method employed was that of Chabbert (2), which consists, briefly, in sowing the different germs over "Agar Muller-Hilton 3.5%", after previous sterilization and observing the zones of growth inhibition around discs of Whatman no. 1 paper impregnated with different concentrations of the compounds to be tested. The measurement of results was taken after 24 h of incubation at 37°. For the development of the fungus Agar Sabouraud was used, incubating at 30° until the control growth was positive.

### 4) Preliminary pharmacodynamic screening

(Carried out only with vespertilin).



a) *In vitro* test.

Undertaken according to (15) on the guinea-pig ileum (Tyrode, 37°), rat uterus (De Jalón, 35°) and rat vas deferens (Krebs, 31°), observing the effect of the steroid against several agonists.

b) *In vivo* methods.

i) Studies of psychoactivity and acute toxicity. These were carried out on ICR Swiss male mice (weight:  $28 \pm 3$  g) according to the Irwin scheme of polydimensional observation (11). The product was suspended in gum arabic 1%, and was administered interperitoneally in increasing doses (50-400 mg/kg), responses being evaluated at different intervals (15', 30', 60').

ii) Cardiovascular activity: performed on male cats anaesthetized with a mixture of chloralose (80 mg/kg) and pentobarbital (5 mg/kg) i.p., and maintained with artificial respiration. The measurement of carotid arterial pressure was taken with a Strathan transducer and the compound (1-25 mg/kg) administered as described above.

## Results and discussion

It may be deduced from the data on cytostatic activity presented in Table I that the steroidal sapogenins investigated in this work are not, on the whole, very active as cell growth inhibitors, inasmuch as only vespertilin, which possesses a lactonic group, attains an interesting value, below that recommended by the Protocols of the National Cancer Institute (5) for more sophisticated tests to be carried out. It must be borne in mind that, while the  $ID_{50}$  of vespertilin proved to be approximately 50 times less active than that of 6-mercaptopurine, used as reference, the low toxicity and minimal pharmacodynamic effects of this product prompted us to undertake *in vivo* assays. The anthraquinonic compounds were completely ineffective.

TABLE I

Compounds		Yield (%)	$ID_{50}$ ( $\mu$ g/ml)
(I)	Dracogenin	0.05	50
(II)	Crestagenin	0.003 (*)	100
(III)	Isoplexigenin B	0.0022 (**)	> 100
(IV)	Vespertilin	0.003	5
(V)	1,4-Dihydroxy-2-methylanthraquinone	—	> 100
(VI)	1-Hydroxy-3-methylanthraquinone	—	> 100
(VII)	1-Hydroxy-2-methyl-4-methoxy-anthraquinone	—	> 100
	6-Mercaptopurine	—	0.1

(\*) From *Isoplexis canariensis*.

(\*\*) From *Semele androgyna*.



Table II presents the antibacterial activity, which may globally be classified as slight, activity being recorded only against Gram + germs and at the highest doses. Again, the lactonic steroid proved the most active.

TABLE II  
Antimicrobial activity.

Compounds	Gram (+) germs			Gram (—) germs		
	B. S.	St. Au.	Str. Fc.	Ps. Ag.	Prot.	Candida albicans
(I) Dracogenin	P (*) (5) (**)	R	R	R	R	0.5 (***)
(II) Crestagenin	P(5)	R	R	R	R	1
(III) Isoplexigenin B	P(5)	R	R	R	R	1
(IV) Vespertilin	P(5)	P(5)	P(5)	R	R	0.5

B. S.: (*Bacillus Sphaericus*); St. Au.: (*Staphylococcus Aureus*); Str. Fc.: (*Streptococcus Faecalis*); Ps. Ag.: (*Pseudomonas Aeruginosa*); Prot.: (*Proteus Sp.*).

(\*) Zone of inhibition in mm.

R (Resistant)  $\leq$  11 mm; P (Partially sensitive: 12-18 mm).

(\*\*) Concentration on the disc (mg/ml).

(\*\*\*) Minimum active concentration (mg/ml).

Almost all the compounds studied showed a marked antifungal effect which might well have proved more intense if, according to the works of Tschesche (18) and Stoessl (16), they had been tested in the form of their corresponding heterocyclic derivatives.

The pharmacological screening carried out exclusively on vespertilin, which yielded the most interesting results of all the compounds in the chemotherapeutic experiments, showed this compound to possess certain antihistaminic and adrenergic effects, judging by the assays undertaken on isolated organs and arterial pressure, and that these effects are present at doses which do not modify the responses induced by the other agonists. With regard to cardiovascular activity, it must be added that no lasting hemodynamic effects were observed at the highest dose administered.

Moreover, vespertilin proved to be only slightly toxic, with  $ID_{50} > 500$  mg/kg i.p., and at doses of 125 and 250 mg/kg i.p. induced a tranquillizing effect, evidenced by passivity characterized by a decrease in spontaneous motor activity, diminished response to external stimuli and irritability. At high doses only, it caused ataxia and slight transitory convulsions in some animals.

### Conclusions

The experiments reported here point out the pharmacobiological potential of vespertilin, a steroidal lactone obtained from *Solanum vespertilio* and never studied before. *S. vespertilio* has proved very rich in



this type of compounds. This work confirms previous experiments on the antitumoral (13) and antifungal (12) effects of this type of compounds.

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